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*Coronavirus Pandemic*

# Tocilizumab treatment of COVID-19: relevance of delay in therapy initiation in middle-income countries

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## Abstract

Introduction: During the coronavirus disease 2019 (COVID-19) pandemic, low- and middle- income countries had less access to monoclonal antibodies, such as tocilizumab (TCZ), compared to high-income countries. This retrospective cohort study aimed at evaluating the impact of a delayed TCZ administration on patient outcomes, and at determining the optimum timing of TCZ initiation for COVID-19 pneumonia in Serbia.

Methodology: The study included 150 patients who received TCZ at a tertiary referral center. The outcomes analyzed in this study were the need for an intensive care unit (ICU) treatment and mortality.

Results: The multiple Cox proportional hazard model suggested that the delay in TCZ administration was an independent predictor of needing ICU treatment and mortality. The receiver operating characteristic (ROC) curve showed that patients who received TCZ after 7.5 days since the onset of symptoms had 74.4% higher chances of needing ICU treatment. Receiving TCZ after 9.5 days since the onset of symptoms, increased the chances of mortality by 78.9%. The multiple Cox proportional hazard model suggested that TCZ administration after 7.5 days since the onset of symptoms increased the hazard for ICU admission by 24.5%; and the hazard of mortality increased by 46.1% after 9.5 days since the onset of symptoms.

Conclusions: This study emphasizes the importance of timely administration of TCZ in COVID-19 pneumonia. Better outcomes were observed when TCZ was administered up to 7.5 days since the onset of symptoms.

**Key words:** COVID-19; tocilizumab; delay; mortality; intensive care unit.

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## Introduction

Progression of coronavirus disease 2019 (COVID-19) involves several underlying factors that lead to organ injury, such as cytokine release, septic shock, thrombosis, and oxidative stress [1–3]. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) binds to angiotensin-converting enzyme 2 (ACE-2) receptors, leading to the activation of Th1 lymphocytes and subsequent production of proinflammatory cytokines, such as interleukin-6 (IL-6) and granulocyte colony-stimulating factor (GM-CSF) [4-6]. Tocilizumab (TCZ) is a recombinant humanized monoclonal antibody that inhibits IL-6 receptors and subsequently lowers the serum IL-6 levels [7]. TCZ has been included in various local, national, and international protocols for COVID-19 treatment; and its efficacy and safety have been recognized in many randomized controlled trials [8,9]. Based on the European Medicines Agency recommendations, the use of TCZ was approved in adults with severe COVID-19 in December 2021 [10].

TCZ use is indicated in patients with severe COVID-19 pneumonia which is characterized by elevated inflammatory markers, such as the C-reactive protein (CRP) and IL-6 [11,12]. TCZ is also warranted in patients who have a rapidly progressing respiratory failure, extensive lung infiltration, and systemic inflammation [8]. In line with the World Health Organization (WHO) recommendations, the use of TCZ is indicated when the IL-6 serum levels exceed 40 pg/mL, or when there is a three-fold increase in the inflammatory marker levels within one day, as well as when respiratory failure is imminent [8,13]. The anticipated duration of TCZ therapy for COVID-19

lasts for one day, with a 12-hour interval between two doses. The recommended dose of TCZ is 8 mg/kg with a maximum single dose of 800 mg [8,10,13].

The key issue surrounding the TCZ treatment is related to the timing of administration. The right timing of TCZ administration is crucial for clinical outcomes, as it is expected to prevent the cytokine storm during COVID-19. Once peak of SARS-CoV-2 infection is established, it may be resistant to further treatment. Therefore, a delayed introduction of TCZ may result in missed opportunities to prevent potentially fatal outcomes [14]. Further, economic development may have influenced the supply and access to TCZ. Based on the gross domestic product (GDP) per capita, Serbia is categorized as a middle-income country. It was observed early on during the pandemic that low- and middle-income countries had reduced access to monoclonal antibodies for COVID-19 therapy compared to their high-income counterparts [15,16]. Despite having repurposed many hospitals to care for COVID-19 patients and having mobilized all healthcare workers in the clinical settings to treat people who needed hospitalization in Serbia, access to TCZ may have been one of the underlying reasons for poorer clinical outcomes of COVID-19.

The objectives of this study were: 1) to investigate predictors of poor prognosis and outcomes of severe COVID-19 in patients treated with TCZ; 2) to assess whether delaying TCZ administration is linked to adverse COVID-19 outcomes; and 3) to determine the optimal timing for TCZ administration in patients with COVID-19 pneumonia at a tertiary referral center in Serbia.

## Methodology

*Setting and participants*

This retrospective cohort study was conducted at the Clinic of Infectious and Tropical Diseases, University Clinical Center of Serbia (UCCS) in Belgrade. The study included a cohort of patients who received TCZ from 1 March 2021 to 7 April 2022. The inclusion criteria were SARS-CoV-2 infection confirmed by reverse transcriptase polymerase chain reaction (RT-PCR), age ≥18 years, and having received TCZ. All patients who received TCZ also received corticosteroids.

The size of the study sample was based on the size of population (approximately 1.6 million residents in the Belgrade metropolitan area), the highest likelihood of COVID-19 positive people who need hospital treatment of 9% as per the literature [17], confidence interval of 95%, and likelihood of alpha error of 5%. The minimum sample size calculated using an online sample size calculator [18] with the aforementioned parameters was 126 participants.

This study complied with the Declaration of Helsinki and was approved by UCCS Ethics Committee (No. 82/3-2023, February 2023). Upon admission, patients signed an informed consent for all diagnostic and therapeutic procedures as well as retrospective studies that would analyze their existing medical records. The study is not registered in a trial registry as it is an observational study.

*Data collection*

Demographic, clinical, and laboratory data were collected from electronic medical records. The demographic data included: gender, age, medical history, initial COVID-19 symptoms, SARS-CoV-2 vaccination status, main complaints and physical findings upon admission, and presence of chronic illnesses. The laboratory parameters were recorded on hospital admission (prior to TCZ administration). The laboratory parameters included red blood cell count (RBC), hemoglobin level (Hgb), white blood cell count (WBC), neutrophil count (Ne), lymphocyte count (Ly), monocyte count (Mo), eosinophil count (Eo), basophil count (Ba), platelet count (PLT), glycemia, CRP, procalcitonin, serum albumin (Alb) levels, IL-6, sodium (Na), potassium (K), lactate dehydrogenase (LDH), D-dimer, ferritin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, gamma-glutamyl transferase (GGT), creatine kinase (CK), prothrombin time (PT), and international normalized ratio (INR). The normal range for IL-6 was defined as < 7 pg/mL.

Upon admission, patients also underwent a chest computed tomography (CT) scan. The severity of COVID-19 pneumonia was measured based on a CT severity score which was determined and analyzed for each patient. The CT severity score was calculated by summing the scores of individual lung lobes involvement and ranged from 0 (no involvement) to 25 (maximum involvement). A score above 75% suggested extensive changes consistent with interstitial pneumonia [19,20].

The timing of TCZ therapy initiation was registered for every patient and assessed as the number of days that passed from the onset of COVID-19 symptoms until the administration of TCZ. The observed outcomes were the rate of transfer to intensive care unit (ICU) and the rate of fatal outcomes in the ICU.

All deceased patients passed away during ICU treatment, and their death was certified by attending

physicians. The attending physician issued a death certificate for each deceased patient, which is a legally binding document in the Republic of Serbia and remains accessible in medical records [21]. For all deceased patients, the immediate cause of death, preceding cause of death, and underlying diseases were defined based on the assessment of the physician who issued the death certificate [21]. The immediate cause of death in all patients included in this study was COVID-19.

*Data analysis*

The patients treated with TCZ were categorized into two groups based on whether they were treated in the ICU or not. Within the ICU-treated group, further subdivision was made based on the survival outcome, distinguishing between patients who died and those who were discharged from the ICU. It is important to emphasize that all deceased patients were treated in the ICU, and thus the mortality of the entire patient sample were assessed in this manner.

Descriptive and analytical statistical methods were applied in this study using the IBM SPSS Statistics software version 17.1 (IBM Corp, Chicago, USA). A $p$ value of $< 0.05$ was considered statistically significant. Descriptive characteristics were presented using means and standard deviations for the continuous variables, while frequencies and percentages were used to describe categorical variables.

The normality of distribution of continuous variables was assessed using the Kolmogorov-Smirnov test. The differences of normally distributed continuous variables were analyzed using the independent samples t tests, whereas non-normally distributed variables were analyzed using the nonparametric two-tailed Mann-Whitney test. The categorical variables were assessed

**Table 1.** Demographic, clinical and laboratory characteristics of the study cohort according to ICU treatment.

| Variable | | Needing ICU treatment | | *p* |
|---|---|---|---|---|
| | | **No N = 111 n (%)** | **Yes N = 39 n (%)** | |
| Gender | Male, n (%) | 35 (23.3%) | 14 (9.3%) | 0.814 |
| | Female, n (%) | 76 (50.7%) | 25 (16.6%) | |
| Age (mean ± SD) years | | 54.1 ± 11.2 | 61.1 ± 12.5 | **0.021** |
| COVID-19 vaccination | Yes, n (%) | 34 (22.7%) | 4 (2.7%) | **0.012** |
| | No, n (%) | 77 (51.3%) | 35 (22.3%) | |
| Timing of TCZ therapy in days, (mean ± SD) | | 5.50 (3.0–6.5) | 8.19 (5.0–12.5) | **0.001** |
| Hypertension, n (%) | | 56 (37.1%) | 19 (12%) | 0.519 |
| Diabetes mellitus, n (%) | | 11 (7.33%) | 18 (12%) | 0.080 |
| Insulin dependent diabetes, n (%) | | 5 (3.33%) | 3 (2%) | **0.010** |
| Oral antidiabetics, n (%) | | 6 (4%) | 12 (8%) | 0.410 |
| Obesity, n (%) | | 6 (4%) | 6 (4%) | **0.040** |
| Malignant tumors, n (%) | | 6 (4%) | 9 (6%) | 0.170 |
| Respiratory diseases, n (%) | | 6 (4%) | 7 (4.66%) | 0.070 |
| Chronic kidney disease, n (%) | | 4 (2.6%) | 1 (0.7%) | 0.615 |
| Duration of symptoms, n (%) | | 19.0 (15.0–23.0) | 23.0 (13.0–30.0) | 0.752 |
| Severity CT score | | 12.36 ± 4.99 | 16.59 ± 5.56 | **0.001** |
| Duration of hospital stay | | 11.0 (9.0–16.0) | 18.0 (7.0–23.0) | 0.557 |
| Red blood cells [$10^{12}$/L] | | 4,94 ± 0,61 | 4.52 ± 0.57 | 0.757 |
| Hemoglobin [g/L] | | 134.0 (122.0–146.0) | 135.0 (129.7–145.7) | 0.546 |
| White blood cells [$10^9$/L] | | 5.40 (4.10–7.40) | 6.20 (4.10–8.30) | 0.447 |
| Lymphocytes [$10^9$/L] | | 0.92 (0.65–1.21) | 1.01 (0.69–1.10) | 0.368 |
| Neutrophils [$10^9$/L] | | 3.85 (2.60–6.05) | 4.72 (2.66–6.12) | 0.747 |
| D-dimer [mg/L] | | 0.63 (0.40–0.95) | 0.73 (0.46–1.31) | 0.162 |
| Prothrombin time [seconds] | | 12.9 (12.2–13.5) | 13.2 (12.25–14.15) | 0.076 |
| INR | | 1.08 (1.02–1.12) | 1.10 (1.02–1.17) | 0.195 |
| Glycemia [mmol/L] | | 6.80 (5.7–8.1) | 7.20 (5.77–8.40) | 0.352 |
| Creatinine [umol/L] | | 85.0 (74.0–101.5) | 90.5 (75.7–116.3) | 0.181 |
| Urea [mmol/L] | | 5.40 (4.40–7.70) | 6.20 (4.85–9.10) | 0.101 |
| Albumin [g/L] | | 32.0 (30.0–35.0) | 33.0 (30.0–37.0) | 0.747 |
| Procalcitonin [ng/mL] | | 0.16 (0.09–0.25) | 0.10 (0.06–0.62) | 0.310 |
| Lactate dehydrogenase [U/L] | | 240.0 (206.0–302.0) | 268.0 (214.0–334.0) | 0.180 |
| C-reactive protein [mg/L] | | 60.02 (26.30–98.60) | 59.50 (26.60–152.20) | 0.430 |
| Ferritin [g/L] | | 568.1 (307.0–1228.0) | 521.0 (300.0–1023.9) | 0.689 |
| Alanine aminotransferase [U/L] | | 39.5 (28.0–62.7) | 46.50 (28.7.0–59.25) | 0.980 |
| Aspartate aminotransferase [U/L] | | 35.0 (24.7–46.0) | 42.0 (25.0–56.5) | 0.143 |
| Gamma glutamyl-transferase [U/L] | | 35.0 (26.0–46.0) | 33.0 (23.0–49.00) | 0.375 |
| Creatine kinase [U/L] | | 116.0 (67.50–244.00) | 224.0 (92.5–433.5) | **0.022** |
| Interleukin 6 [pg/mL] | | 47.2 (29.6–74.4) | 83.60 (36.0–185.0) | **0.006** |

COVID-19: coronavirus disease 2019; CT: computerized tomography; ICU: intensive care unit; IND: - international normalized ratio; SD: standard deviation; TCZ: tocilizumab. Values in **bold** are statistically significant.

using the Fisher's exact test (when there were < 5 observations per cell) or the Chi square test.

The Cox proportional hazard model was used to identify factors associated with needing ICU treatment and mortality. All demographic, clinical and laboratory parameters were tested first in a univariate model. All univariately significant characteristics were analyzed in the multivariate model.

The time to TCZ administration was analyzed using the receiver operating characteristic (ROC) curve to better understand the likelihood of transfer to ICU and dying in ICU, based on the area under curve (AUC) and the level of sensitivity and specificity. The cut-off values of the time elapsed from symptom onset to the initiation of TCZ treatment in days were defined, using ROC analysis, for outcome differentiation. Additionally, the significance of this finding was examined through regression analysis.

The Kaplan-Meier survival curve was used to analyze survival of patients who received TCZ before and after the cut-off time observed in the ROC curve.

## Results

A total of 150 patients were included in the study. The majority were male (101; 67.3%). The average age of the patients was 62.36 ± 13.21 years. Most patients were not vaccinated (112; 74.7%). On average, TCZ therapy was started 8.26 ± 2.76 days after the onset of symptoms of COVID-19.

The majority of patients treated in the ICU were older and unvaccinated. The ICU patients also exhibited significantly higher serum levels of IL-6 (47.2 vs. 83.6, $p$ = 0.006) and CK (116.0 vs. 224.0, $p$ = 0.022) compared to non-ICU patients. However, no significant differences were observed in other laboratory parameters before and after the administration of TCZ.

**Table 2.** Demographic, clinical and laboratory characteristics of the study cohort according to ICU mortality.

| Variable | | Died in the ICU | | *p* |
|---|---|---|---|---|
| | | **No N = 131 n (%)** | **Yes N = 19 n (%)** | |
| Gender | Male, n (%) | 89 (59.3%) | 11 (7.3%) | 0.373 |
| | Female, n (%) | 42 (28.0%) | 8 (5.3%) | |
| Age (mean ± SD) years | | 63.1 ± 14.6 | 62.2 ± 13.0 | 0.651 |
| COVID-19 vaccination | Yes, n (%) | 35 (23.3%) | 2 (1.3%) | 0.116 |
| | No, n (%) | 96 (64%) | 17 (11.3%) | |
| Timing of TCZ therapy | | 6.0 (4.5-8.0) | 10.5 (8.0–14.0) | **0.001** |
| Timing of TCZ therapy in days, (mean ± SD) | | 66 (43.7%) | 9 (6.0%) | 0.512 |
| Hypertension, n (%) | | 3 (2.0%) | 5 (3.3%) | **0.001** |
| Diabetes mellitus, n (%) | | 12 (7.9%) | 6 (4.0%) | **0.013** |
| Insulin dependent diabetes, n (%) | | 7 (4.6%) | 5 (3.3%) | **0.008** |
| Oral antidiabetics, n (%) | | 4 (2.6%) | 11 (7.3%) | 0.094 |
| Obesity, n (%) | | 3 (2.0%) | 10 (6.6%) | 0.238 |
| Malignant tumors, n (%) | | 5 (3.5%) | 0 (0.0%) | 0.456 |
| Respiratory diseases, n (%) | | 19.0 (15.0–24.0) | 23.0 (13.0–30.0) | 0.347 |
| Chronic kidney disease, n (%) | | 11.0 (9.0–16.0) | 18.0 (7.0–23.0) | 0.475 |
| Duration of symptoms, n (%) | | 12.42 ± 5.07 | 19.59 ± 3.41 | **0.001** |
| Severity CT score | | 4.55 (4.20–4.85) | 4.41 (4.10–4.88) | 0.469 |
| Duration of hospital stay | | 130.0 (120.0–148.0) | 135.0 (125.0–146.0) | 0.635 |
| Hemoglobin [g/L] | | 6.10 (3.80–8.3) | 5.60 (4.10–7.80) | 0.980 |
| White blood cells [$10^9$/L] | | 0.95 (0.67–1.24) | 0.91 (0.63–1.10) | 0.528 |
| Lymphocytes [$10^9$/L] | | 3.89 (2.66–6.07) | 4.59 (2.41–6.37) | 0.975 |
| Neutrophils [$10^9$/L] | | 0.64 (0.40–0.91) | 0.66 (0.47–1.46) | 0.270 |
| D-dimer [mg/L] | | 12.9 (12.2–13.4) | 13.60 (12.50–14.30) | **0.021** |
| Prothrombin time [s] | | 1.08 (1.02–1.12) | 1.12 (1.04–1.18) | 0.066 |
| INR | | 6.90 (5.7–8.1) | 7.40 (5.70–8.40) | 0.467 |
| Glycemia [mmol/L] | | 85.0 (75.0–102.5) | 83.0 (72.0–117.0) | 0.721 |
| Creatinine [umol/L] | | 5.80 (4.65–7.75) | 6.00 (4.10–8.42) | 0.944 |
| Urea [mmol/L] | | 33.0 (30.0–36.0) | 32.0 (30.0–36.0) | 0.747 |
| Albumin [g/L] | | 0.16 (0.09–0.25) | 0.10 (0.06–0.62) | 0.827 |
| Procalcitonin [ng/mL] | | 240.0 (204.0–307.0) | 281.0 (218.5–358.0) | 0.721 |
| Lactate dehydrogenase [U/L] | | 59.7 (25.6–98.5) | 96.70 (38.55–186.40) | **0.048** |
| C-reactive protein [mg/L] | | 529.1 (292.1–979.1) | 626.7 (439.9–1405.8) | 0.187 |
| Ferritin [g/L] | | 39.5 (28.0–59.7) | 51.0 (30.0–60.5) | 0.354 |
| Alanine aminotransferase [U/L] | | 35.0 (24.2–46.0) | 42.0 (28.0–66.5) | 0.142 |
| Aspartate aminotransferase [U/L] | | 35.5 (26.6–53.0) | 33.0 (22.0–81.00) | 0.604 |
| Gamma glutamyl-transferase [U/L] | | 69.0 (129.0–247.0) | 263.5 (98.5–426.7) | **0.046** |
| Creatine kinase [U/L] | | 49.1 (29.7–76.0) | 140.0 (45.7–245.7) | **0.001** |
| Interleukin 6 [pg/mL] | | | | |

COVID-19: coronavirus disease 2019; CT: computerized tomography; ICU: intensive care unit; INR: - international normalized ratio; SD: standard deviation; TCZ: tocilizumab. Values in **bold** are statistically significant.

Moreover, patients treated in the ICU exhibited higher values of the CT score for COVID-19 pneumonia (12.36 vs. 16.59, $p = 0.001$), indicating a greater extent of lung involvement (Table 1).

Patients who died in the ICU following TCZ administration exhibited significantly elevated levels of IL-6 (49.1 vs. 140.0, $p = 0.001$), CRP (59.7 vs. 96.70, $p = 0.048$), and CK (69.0 vs 263.5, $p = 0.046$) as well as shorter prothrombin time compared to those who recovered (12.9 vs 13.6, $p = 0.021$). Furthermore, patients who experienced fatal outcomes during ICU treatment had higher values of the CT score for COVID-19 pneumonia (11.0 vs. 18.0, $p = 0.001$) (Table 2).

*Transfer to the ICU*

During hospital stay, 39 (26%) COVID-19 patients were transferred to the ICU, following the TCZ administration, while the remaining 111 (74%) patients were successfully treated in general wards. Baseline characteristics of patients are presented in Table 1. Subjects treated in the ICU were on average older and less likely to be vaccinated against COVID-19, and had a higher CT severity score of COVID-19 pneumonia as well as higher levels of CK and IL-6, compared to those treated in the general wards. Patients who continued treatment in the ICU were more often obese and had insulin-dependent diabetes. Also, the ICU group received TCZ later compared to the non-ICU group (Table 1).

*Outcomes in the ICU*

Of the 39 patients who required ICU treatment, a total of 19 died following the administration of TCZ, resulting in a mortality rate of 12.7%. All deceased patients were treated in the ICU. Baseline characteristics of patients' vital status in the ICU are shown in Table 2. Patients who died in the ICU had a higher CT severity score of COVID-19 pneumonia, prothrombin time, CRP, CK, and IL-6 levels compared to those who were discharged from the ICU. Patients who died in the ICU were more often obese and had diabetes. In addition, people who died in the ICU received TCZ at a later day compared to survivors (Table 2).

*Predictors of transfer to the ICU*

The Cox proportional hazard model showed that a shorter hospital stays, delay in TCZ administration, obesity, lower lymphocyte count, and higher levels of creatine kinase were univariately associated with receiving ICU treatment (Supplementary Table 1). These univariately significant variables were, therefore, tested together in the multivariate model. The multivariate model showed that a delay in TCZ treatment was an independent predictor of transfer to the ICU (Table 3).

*Predictors of ICU mortality*

The same univariate model showed that shorter duration of hospital stays; delay in TCZ treatment initiation; higher severity CT score of COVID-19 pneumonia; diabetes; obesity; lower lymphocyte count; and higher levels of CK, IL-6 and LDH were univariately associated with ICU mortality (Supplementary Table 1). These univariately significant variables were, therefore, tested together in the multivariate model. The multivariate model showed that longer hospital stays, delay in TCZ treatment initiation, higher severity CT score were independent predictors of ICU mortality (Table 3).

*Analysis of the impact of delay in TCZ therapy*

Given that the delay in TCZ administration was predictive of receiving the ICU treatment as well as ICU mortality, we analyzed it in more detail using the ROC curve. The AUC > 0.7 suggested that a delay in TCZ initiation was able to discriminate patients who were likely to transfer to ICU and die in the ICU. Specifically, patients who received TCZ after day 7.5

**Table 3.** Results of the multivariate Cox proportional hazard model: factors associated with receiving ICU treatment and mortality in the ICU.

| Variable | Receiving ICU treatment | | | Mortality in ICU | | |
|---|---|---|---|---|---|---|
| | HR | 95% CI | *p* | HR | 95% CI | *p* |
| Timing of TCZ therapy | 1.201 | 1.07–1.39 | **0.004** | 1.210 | 1.02–1.73 | **0.035** |
| Severity CT score | | | | 1.325 | 1.06–1.83 | **0.026** |
| Insulin dependent diabetes | | | | 0.240 | 0.01–6.96 | 0.301 |
| Oral antidiabetics | | | | 0.191 | 0.01–5.98 | 0.325 |
| Obesity | 0.491 | 0.19–1.276 | 0.101 | 0.314 | 0.03–2.82 | 0.261 |
| Lymphocytes [$10^9$/L] | 1.062 | 0.90–1.25 | 0.451 | 0.642 | 0.05–8.20 | 0.721 |
| Interleukin 6 [pg/mL] | | | | 0.990 | 0.99–1.01 | 0.304 |
| Creatine kinase [U/L] | 1.001 | 1.00–1.00 | 0.210 | 1.001 | 0.98–1.01 | 0.904 |
| Lactate dehydrogenase [U/L] | | | | 0.998 | 0.99–1.01 | 0.391 |

CI: confidence interval; CT: computerized tomography; ICU: intensive care unit; HR: hazard ratio; TCZ: tocilizumab. Values in **bold** are statistically significant.

**Table 4.** Parameters of the receiver operating characteristics (ROC) curve analysis.

| ICU mortality as an outcome variable | | | |
|---|---|---|---|
| **Variable** | **Area under the curve** | **Standard error** | ***p*** |
| | 0.831 | 0.061 | 0.001 |
| Day of receiving TCZ | Cut-off | Sensitivity | Specificity |
| | 9.5 days | 78.9% | 79.4% |
| **ICU treatment requirement** | | | |
| **Variable** | **Area under the curve** | **Standard error** | ***p*** |
| | 0.737 | 0.051 | 0.001 |
| Day of receiving TCZ | Cut-off | Sensitivity | Specificity |
| | 7.5 days | 74.4% | 59.5% |

ICU: intensive care unit; TCZ: tocilizumab.

since the onset of COVID-19 symptoms had 74.4% chances of being treated in the ICU (Figure 1B, Table 4). Similarly, a delay in TCZ initiation showed discriminative power (AUC > 0.7) in prediction of ICU mortality. Specifically, patients who received TCZ after 9.5 days since the onset of COVID-19 symptoms had 78.9% chance of dying in the ICU (Figure 1A, Table 4).

Regression analysis suggested that patients who received TCZ 7.5 days after the onset of COVID-19 symptoms had a 24.5% increased likelihood of ICU admission (HR: 1.245, 95% CI: 1.10–1.62, $p = 0.024$), while a high CT score of pneumonia at admission was associated with an 18% increased likelihood of ICU admission (HR: 1.080, 95% CI: 1.01–1.16, $p = 0.032$) (Table 5, Model 1). Similarly, patients who received TCZ after 9.5 days from symptom onset had a 46.1% increased likelihood of mortality (HR: 1.461, 95% CI: 1.25–1.90, $p = 0.002$), while a high CT score of pneumonia at admission was associated with a 32% increased likelihood of ICU admission (HR: 1.320, 95% CI: 1.25–1.90, $p = 0.002$) (Table 5, Model 2).

*Comparative survival based on the delay in TCZ administration*

We observed that delay in TCZ treatment may have contributed to the ICU mortality in our cohort. Therefore, we analyzed the Kaplan Meier curve to assess the survival of patients who received TCZ before and after day 9.5 since the onset of COVID-19 symptoms. Based on a predefined threshold of 9.5 days, patients were divided into two groups: 1) those who received TCZ within 9.5 days from the onset of symptoms and 2) those who received TCZ after day 9.5 from the onset of symptoms. The Kaplan-Meier survival curve suggested a statistically significant difference in mortality (log rank test $p < 0.001$).

**Figure 1.** Receiver operating characteristic curve analysis to differentiate coronavirus disease 2019 (COVID-19) patients (**A**) who needed intensive care unit (ICU) treatment and (**B**) who died in the ICU based on the delay in tocilizumab administration

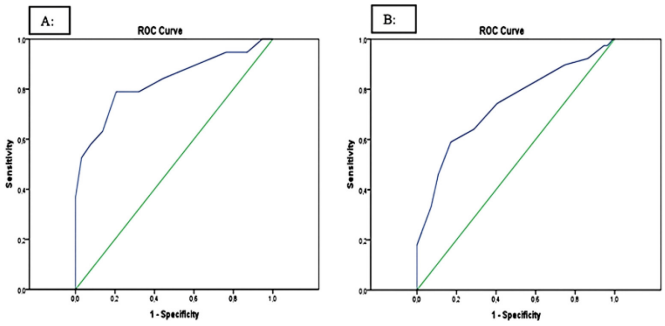


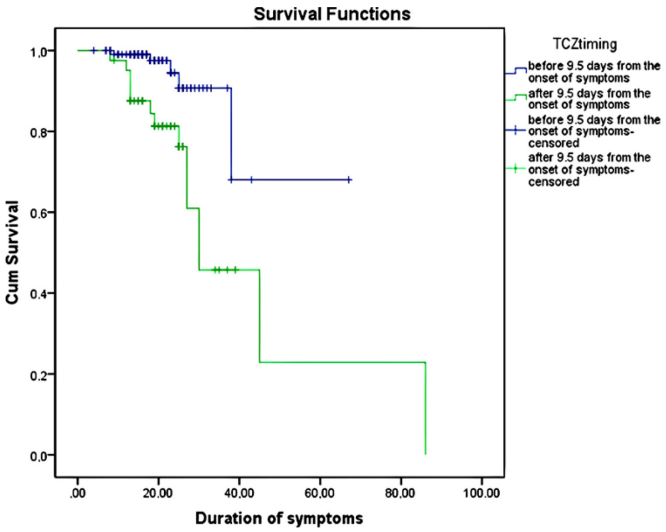

**Table 5.** Results of the Cox proportional hazard model; model 1: outcome variable—continuation of treatment in ICU; model 2: outcome variable—mortality

| **Model 1** | **Univariate Cox model** | | | **Multivariate Cox model** | | |
|---|---|---|---|---|---|---|
| | **HR** | **95% CI** | ***p*** | **HR** | **95% CI** | ***p*** |
| Age | 1.014 | 0.98–1.04 | 0.355 | | | |
| Gender | 0.662 | 0.34–1.29 | 0.227 | | | |
| COVID-19 vaccination | 0.467 | 0.16–1.325 | 0.152 | | | |
| Severity CT score | 1.064 | 0.99–1.14 | 0.093 | 1.180 | 1.01–1.16 | **0.032** |
| Day of receiving TCZ > 7.5 | 1.210 | 1.02–1.54 | **0.012** | 1.245 | 1.10–1.62 | **0.024** |
| Day of receiving TCZ < 7.5 | 0.752 | 0.35–0.94 | 0.251 | | | |
| **Model 2** | **Uniivariate Cox model** | | | **Multivariate Cox model** | | |
| | **HR** | **95% CI** | **p** | **HR** | **95% CI** | ***p*** |
| Age | 0.988 | 0.95–1.03 | 0.536 | | | |
| Gender | 0.449 | 0.17–1.17 | 0.102 | 0.523 | 0.19–1.21 | 0.069 |
| COVID-19 vaccination | 0.511 | 0.116–2.24 | 0.511 | | | |
| Severity CT score | 1.253 | 1.10–1.41 | **0.001** | 1.320 | 1.21–1.37 | 0.001 |
| Day of receiving TCZ > 9.5 | 1.453 | 1.23–1.84 | **0.001** | 1.461 | 1.25–1.90 | **0.002** |
| Day of receiving TCZ < 9.5 | 0.651 | 0.20–0.98 | 0.152 | | | |

CI: confidence interval; COVID-19: coronavirus disease 2019; HR: hazard ratio; TCZ: tocilizumab. Values in **bold** are statistically significant.

This means that patients who received TCZ after day 9.5 from the onset of COVID-19 symptoms had poorer survival compared to patients who received TCZ within 9.5 days of symptoms onset (Figure 2).

**Figure 2.** Comparative survival of patients who received tocilizumab within 9.5 days of symptoms onset and after 9.5 days since the symptoms onset (log rank test for difference: $p < 0.001$).




## Discussion

This study underscores the necessity of initiating TCZ treatment promptly, which has been identified in previous research as an effective intervention in the treatment of COVID-19 [7,9]. Overall, the study findings suggest that patients who had an unfavorable disease trajectory were hospitalized for a longer period of time and received TCZ with a delay, compared with those who were successfully treated. Patients who died in the ICU had higher levels of IL-6, CK, and CRP, along with longer prothrombin time than those who were discharged. Shorter hospital stays, delay in TCZ administration, and CT score of COVID-19 pneumonia were independent predictors of ICU mortality. Initiation of TCZ therapy by day 9 of symptoms onset was identified as the optimum treatment time to improve survival of COVID-19 patients.

A previous meta-analysis suggested that an early initiation of TCZ, within the initial 24–48 hours of hospital admission, reduces mortality and improves clinical recovery in severe COVID-19 patients [20]. Studies assessing the impact of TCZ on 28-day mortality suggest its efficacy, particularly when administered at the point of $PaO_2/FiO_2 < 200$, leading to enhanced survival and improved clinical outcomes in severe COVID-19 patients [22]. Nonetheless, there are still conflicting data regarding the optimal timing for TCZ treatment, as certain studies suggest that delay in treatment initiation, such as the administration at the later stages of COVID-19, may offer greater benefits and prevent tissue damage. Thus, authors have reported that a delayed administration of TCZ following the initial peak of viral replication resulted in a more effective modulation of the immune response and a decreased risk of secondary infections [23]. On the other hand, the REMAP-CAP trial has shown that the administration of TCZ in the first 24 hours after ICU admission was associated with a 59% increase in chances of 90-day survival [24]. These results suggest that the greatest benefits of TCZ are observed when it is administered after viral replication, but prior to development of organ dysfunction, potentially allowing for a reversal of the looming cytokine storm.

Some evidence indicates that the introduction of TCZ within the initial 48 hours of ICU admission may be associated with a reduced risk of in-hospital mortality among critically ill patients with COVID-19, when compared to those who did not receive early TCZ treatment [25]. In fact, this study highlighted that a delay in TCZ administration significantly affected both ICU admission and ICU mortality rates. Finally, most of available data underline that TCZ is most effective when introduced early in the course of infection, allowing the reversal of potential organ dysfunction [26]. In an observational study conducted in the United States, the beneficial effect of TCZ on mortality was notably observed, especially among patients admitted to the ICU, within 3 days of symptom onset [27]. Similar observational studies have noted a consensus that delaying the initiation of TCZ increases the likelihood of adverse events; however, precise timeframes were lacking [28,29].

Some studies indicated limited efficacy of TCZ on long-term outcomes, although precise data on the timing of therapy initiation were lacking. The CORIMUNO trial, involving 131 hypoxemic patients, did not reveal a significant effect of TCZ on clinical outcomes at 28 days [30]. Similarly, the Boston Area COVID-19 Consortium (BACC) Bay Tocilizumab Trial, which enrolled 242 hypoxemic patients, found no significant difference in the median time to discontinuation of supplemental oxygen between the TCZ and placebo groups [31].

All participants included in our study received treatment consisting of TCZ, corticosteroid therapy, and antivirals. Interestingly, a noteworthy interaction between the IL-6 antagonists and corticosteroids was observed. Specifically, the concurrent administration of corticosteroids and TCZ resulted in a significant decrease in 28-day all-cause mortality when compared

to placebo or standard of care [32]. Furthermore, a combined use of TCZ and corticosteroids was associated with a lower probability of needing mechanical ventilation, extracorporeal membrane oxygenation (ECMO), or dying within 28 days, compared to placebo or standard of care [33]. However, the administration of TCZ alone without corticosteroids did not show appreciable improvements in reduction of rates of poorer outcomes when compared to placebo or standard care [33,34].

Serum biomarkers such as CRP, IL-6, and ferritin have been proposed as potential indicators to guide the timing of TCZ therapy in COVID-19 patients [35]. In this study, a correlation was observed between higher CRP values at therapy initiation and likelihood of needing ICU treatment. This result can be explained by the notion that patients delay to seek help in healthcare institutions, which subsequently delays the TCZ treatment initiation resulting in a more severe disease course. The anticipated impact of TCZ on CRP and neutrophil count stems from its ability to inhibit the IL-6-CRP axis, which plays a pivotal role in both acute and chronic inflammation [36]. This axis influences the expression of the acute-phase proteins, hematopoiesis, response to microbial insults, and debris clearance in tissue injury scenarios. The importance of modulating this axis in early phases of inflammation is particularly emphasized by adequate administration of TCZ [37,38].

The present study has several limitations that should be acknowledged. Firstly, potential influence of different SARS-CoV-2 variants on the course and outcome of COVID-19 was not taken into consideration. During the study period, Beta, Delta, and Omicron variants were the most common in our surroundings. Given that new variants of SARS-CoV-2 may rise and have distinctive characteristics, their impact on treatment response and patient outcomes remains unclear and warrants further research. Secondly, this study was conducted retrospectively and was limited to a single center. Retrospective studies have inherent limitations in terms of data collection and potential biases in patient selection. The findings should be interpreted with caution and validated in larger, prospective studies involving multiple centers to enhance the generalizability of the results. Despite these limitations, this study provides valuable insights into the association between the timing of TCZ therapy and the course and outcome of COVID-19. The observed differences in mortality based on TCZ treatment delay highlight the relevance of early intervention in patients hospitalized due to COVID-19 pneumonia.

## Conclusions

We identified that delay in TCZ administration was associated with needing ICU treatment and mortality. This suggests that TCZ should be administered up to day 9.5 since the onset of COVID-19 symptoms to ensure optimum survival in a single middle income country center. In addition, administration of TCZ up to day 7.5 since the symptom onset may reduce the likelihood of requiring the ICU treatment. Thus, timely administration of TCZ in the management of COVID-19 could be the key to improve disease outcomes and these features should be especially emphasized in future pandemics in middle-income countries.

### Authors contributions

BB, OS, NN, NM, TG, NT, AF, JD, MS, JS, and IM contributed to the study design, data collection, data analysis and interpretation. BB drafted the manuscript; and OS, NN, NM, TG, NT, AF, JD, MS, JS, and IM provided critical review of the manuscript. All authors approve the final version of the manuscript and agree to be held accountable for all aspects of the manuscript.

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### Conflict of interests

No conflict of interests is declared.

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## Annex – Supplementary Items

**Supplementary Table 1.** Results of the univariate Cox proportional hazard model: factors associated with receiving ICU treatment and mortality in the ICU.

| Variable | Receiving ICU treatment | | | Mortality in ICU | | |
|---|---|---|---|---|---|---|
| | HR | 95% CI | *p* | HR | 95% CI | *p* |
| Age | 1.014 | 0.98–1.04 | 0.355 | 0.988 | 0.95–1.03 | 0.536 |
| Gender | 0.662 | 0.34–1.29 | 0.227 | 0.449 | 0.17–1.17 | 0.102 |
| Smoking | 1.319 | 0.59–2.93 | 0.496 | 1.073 | 0.31–3.68 | 0.915 |
| Vaccination status | 0.467 | 0.16–1.325 | 0.152 | 0.511 | 0.116–2.24 | 0.511 |
| Number of vaccine doses | 0.747 | 0.42–1.34 | 0.328 | 0.635 | 0.23–1.76 | 0.353 |
| Length of hospital stay | 0.861 | 0.81–0.92 | **0.001** | 0.848 | 0.77–0.93 | **0.001** |
| Timing of TCZ | 1.106 | 1.01–1.22 | **0.045** | 1.156 | 1.02–1.31 | **0.019** |
| Severity CT score | 1.064 | 0.99–1.14 | 0.093 | 1.253 | 1.10–1.41 | **0.001** |
| Hypertension | 1.695 | 0.88–3.25 | 0.113 | 1.885 | 0.73–4.85 | 0.189 |
| Insulin dependent diabetes | 0.627 | 0.24–1.63 | 0.340 | 0.259 | 0.09–0.75 | **0.013** |
| Oral antidiabetics | 0.680 | 0.28–1.63 | 0.390 | 2.430 | 0.09–0.66 | **0.006** |
| Obesity | 0.326 | 0.13-0.79 | **0.014** | 0.172 | 0.06–0.50 | **0.001** |
| Solid tumor | 0.522 | 0.18–1.48 | 0.224 | 0.496 | 0.11–2.20 | 0.356 |
| Hematological malignancy | 1.102 | 0.15–8.10 | 0.924 | 0.571 | 0.07–4.34 | 0.588 |
| Respiratory diseases | 2.370 | 0.09–6.51 | 0.388 | 1.622 | 0.21–12.69 | 0.645 |
| Chronic kidney disease | 0.890 | 0.12–6.57 | 0.909 | 0.398 | 0.05–3.09 | 0.379 |
| Red blood cells | 0.990 | 0.53–1.85 | 0.975 | 0.991 | 0.52–1.74 | 0.942 |
| Hemoglobin | 1.006 | 0.98–1.03 | 0.575 | 0.948 | 0.41–1.87 | 0.478 |
| Leukocytes | 1.002 | 0.92–1.09 | 0.969 | 0.960 | 0.84–1.09 | 0.558 |
| Lymphocytes | 0.942 | 0.86–1.35 | **0.007** | 0.993 | 0.85–1.28 | **0.012** |
| Neutrophils | 0.993 | 0.91–1.09 | 0.888 | 0.975 | 0.85–1.12 | 0.721 |
| Platelets | 0.999 | 0.99–1.00 | 0.669 | 0.999 | 0.99–1.00 | 0.626 |
| Glycemia | 1.002 | 0.91–1.09 | 0.962 | 0.413 | 0.10–1.63 | 0.208 |
| Urea | 1.026 | 0.94–1.12 | 0.571 | 1.017 | 0.67–1.53 | 0.936 |
| Albumin | 1.044 | 1.01–1.10 | 0.074 | 1.031 | 1.10–1.41 | 0.091 |
| Procalcitonin | 1.012 | 0.21–1,21 | 0.423 | 1.013 | 0.97–1.02 | 0.222 |
| Feritin | 1.000 | 0.99–1.00 | 0.322 | 1.001 | 0.99–1.00 | 0.634 |
| International normalized ratio | 0.968 | 0.83–1.13 | 0.968 | 0.947 | 0.82–0.97 | 0.872 |
| Prothrombin time | 1.072 | 0.98–1.17 | 0.145 | 1.081 | 0.99–1.10 | 0.141 |
| D-dimer | 0.853 | 0.61–1.20 | 0.354 | 0.861 | 0.54–1.37 | 0.526 |
| Interleukin 6 | 1.000 | 0.99-1.00 | 0.615 | 1.002 | 1.00–1.01 | **0.012** |
| Creatine kinase | 1.000 | 1.00–1.02 | **0.010** | 1.001 | 1.00–1.01 | **0.002** |
| C-reactive protein | 1.001 | 0.99–1.05 | 0.778 | 1.005 | 1.00–1.02 | 0.071 |
| Lactate dehydrogenase | 1.001 | 0.99–1.04 | 0.251 | 1.003 | 0.99–1.01 | **0.044** |
| Alanine aminotransferase | 1.001 | 0.99–1.05 | 0.804 | 1.003 | 0.99–1.01 | 0.406 |
| Aspartate aminotransferase | 1.002 | 1.00–1.04 | 0.06 | 1.002 | 0.98–1.01 | 0.080 |
| Gamma glutamyl-transferase | 1.001 | 1.00–1.02 | 0.172 | 1.001 | 1.00–1.01 | 0.090 |

CT: computerized tomography; HR: hazard ratio; ICU: intensive care unit; TCZ: tocilizumab. Values in **bold** are statistically significant at $p < 0.05$.